

Catechin Content and Antioxidant(Tuty Anggraini)

CATECHIN CONTENT AND ANTIOXIDANT ACTIVITY OF SOME BREWED TEMPERATURE OF BLACK TEA SYRUP

Kandungan Katechin Dan Aktifitas Antioksidan Pada Beberapa Temperatur Seduhan Sirup Teh Hitam

Tuty Anggraini

Fakultas Teknologi Pertanian, Universitas Andalas

Kampus Limau Manis, Padang 25163

e-mail: tuty_anggraini@yahoo.co.id

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ABSTRACT

The catechin contents and the antioxidant activity of black tea syrup that brewed at some temperature still poorly understood. It has been investigated whether catechin contents and antioxidant activity of black tea syrup may be affected by hot or cold water. The temperature of 30°C, 60°C, and 90°C were used to brew the syrup, and then the catechin and the antioxidant were determined at each temperature by using DPPH radical scavenging activity method. Measurement of DPPH was conducted for 2 minutes reaction time. Catechin contents were determined by using HPLC method. The result of research showed that the catechin and the antioxidant activity affected by temperature tested. Five kinds of catechin derivatives detected in black tea syrup, EGC (epigallocatechin), C (catechin), EGCG (epigallocatechin gallate), EC (epicatechin) and ECG (epicatechin gallate). The highest DPPH scavenging activity of black tea syrup was showed by 30°C.

Key words: *black tea syrup, antioxidant, DPPH, catechin.*

ABSTRAK

Kandungan katechin dan aktivitas antioksidan dari sirup teh hitam yang diseduh pada beberapa temperatur masih belum begitu dipahami. Pada penelitian ini telah diselidiki apakah kandungan katechin dan aktioksidan dipengaruhi oleh air dingin atau air panas. Temperatur 30°C, 60°C, dan 90°C digunakan untuk menyeduh sirup teh hitam lalu ditentukan kandungan katechin dan aktifitas antioksidannya pada masing-masing temperatur menggunakan metode aktifitas perangkap radikal bebas DPPH (dyphenyl pycrylhydrazyl). Pengukuran DPPH dilakukan selama waktu reaksi dua menit. Kandungan katechin ditentukan menggunakan metode HPLC (High Performance Liquid Chromatography). Hasil penelitian menunjukkan bahwa kandungan katechin dan aktifitas antioksidan sirup teh hitam dipengaruhi oleh temperatur yang diuji. Lima jenis turunan katechin terdeteksi dalam sirup teh hitam yaitu EGC (epigallocatechin), C (catechin), EGCG (epigallocatechin gallate), EC (epicatechin) and ECG (epicatechin gallate). Aktifitas perangkap DPPH tertinggi dari sirup teh hitam diperoleh pada temperatur 30°C.

Key words: *black tea syrup, antioxidant, DPPH, catechin*

INTRODUCTION

Catechins, the main constituents of tea, have an antioxidant potential. The health benefits of tea are primarily attributed to its antioxidant properties and the ability of catechins to scavenge reactive oxygen species (ROS) (Yang, 1999). ROS

capable of causing damage to DNA have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age. Minimizing oxidative damage may be one of the most important approaches to the primary prevention of these aging and health problems. Antioxidants terminate

ROS attacks and appear to be a key component in potential treatment (Yu, 2007).

Tea is obtained from the leaves of the plant *Camellia sinensis* and is a popularly consumed beverage worldwide. Fresh tea leaves contain (on average related to dry substance mass): 36% polyphenolic compounds, 25% carbohydrates, 15% proteins, 6.5% lignin, 5% ash, 4% amino acids, 2% lipids, 1.5% organic acids, 0.5% chlorophyll, and carotenoids and volatile substances constituting less than 0.1% (Harold and Graham, 1992).

One of the most beneficial properties of tea is the antioxidant activity and free radical-scavenging ability of the polyphenol component (Frei and Higdon, 2003). Syrup may be made from black tea as a healthy beverage.

The use of the stable free radical diphenylpicrylhydrazyl (DPPH) is a popular method for estimating antioxidant activity. Antioxidant components are most important in foods because of their ability to reduce free radical-mediated degradation of cells and tissues in an organism (Jin *et al.*, 2004; Wongkham *et al.*, 2001). The main catechin group consists of eight polyphenolic flavonoid-type compounds: catechin (C), epicatechin (EC), galocatechin (GC), epigallocatechin (EGC), catechin gallate (CG), epicatechin gallate (ECG), galocatechin gallate (GCG), and epigallocatechin gallate (EGCG).

Tea can be prepared in a number of ways, but usually it is made into black or green tea. Black tea is obtained by a fermentation process, whereas for making green tea, fermentation is purposely prevented. The black tea production begins with plucking, withering, maceration (rolling), and drying. During withering, the leaves take on a form facilitating the rolling process. This process results in disrupting the cell structure of the leaves and the fermentation process then begins (Bokudava and Skolebeleva, 1980).

Black tea syrup is a viscous liquid containing a large amount of dissolved sugars from black tea infusion. As a beverage, black tea syrup is served in hot or cold conditions. The conditions can contribute in the antioxidant property of

black tea syrup itself, so it is necessary to know the antioxidant activity in black tea syrup for each condition. The temperatures 30, 60 and 90°C are believed to represent cold to warmth conditions for brewing black tea syrup. In this study, the contents of individual catechins in black tea syrup prepared at different temperatures were investigated and the DPPH scavenging activities in black tea syrup at 2 min.

MATERIALS and METHODS

Materials

Black tea was obtained from Mitra Kerinci Plantation in West Sumatra, Indonesia. DPPH was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Ethanol was purchased from Wako Pure Chemical Industries. Epicatechin and catechin were purchased from Wako Chemical Industries, Ltd. Epigallocatechin, epigallocatechin gallate, galocatechin gallate, epicatechin gallate, and catechin gallate were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade solvents were filtered through a 0.20- μ l membrane (Sartorius Biotech GmbH 37070, Goettingen, Germany) and degassed in an ultrasonic bath before use.

Preparation of black tea syrup

Black tea syrup was made by stirring granulated sugar (70°Brix) into hot black tea extract (110 g/l) until the sugar dissolved, the solution was then cooled. The samples used were in liquid form and were brownish in appearance. The ratio of water to black tea syrup used for consumption was 5:1. The black tea syrup was prepared at brew temperatures of 30, 60 and 90°C. These black tea samples were used for the measurement of antioxidant activity. For DPPH measurement, 4 ml of black tea syrup extract was mixed with 2 ml of 200 mM MES buffer, 0.4 ml distilled water, and 1.6 ml of 50% ethanol.

DPPH radical scavenging activity**DPPH of black tea syrup**

DPPH radical scavenging activity was determined according to the method of Blois with slight modification. A 200- μ M solution of DPPH in ethanol was prepared (3.94 mg DPPH in 50 ml 99.5% ethanol), and then 300 μ l of this solution was mixed with 150 μ l of 200 mM MES buffer, 150 μ l distilled water, and (600-a) μ l of 50 mM MES buffer. Final concentrations were 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00, and 5.00 mg/ml (600 ml of total volume). For the control solution, DPPH was substituted with the 99.5% ethanol solution.

For black tea syrup extract measurements, 1 g of black tea syrup sample was diluted with 80% ethanol to a volume of 25 ml. Four milliliters of this extract was added to 1 ml of 200 mM MES buffer, 0.2 ml distilled water, and 0.8 ml of 50% ethanol. The reactions were allowed to proceed for 2 min and 30 min at room temperature.

The absorbance of the samples was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{control absorbance} - \text{extract absorbance}) \times 100}{\text{control absorbance}}$$

Extraction and analysis of black tea syrup

The black tea syrup was extracted with ethanol and then filtered through a 0.20- μ m nylon filter.

Catechins analysis

A Shimadzu (Tokyo, Japan) liquid chromatographic system consisting of a system SCL-10A controller, SPD-10AV UV-VIS detector, LC-10AD liquid chromatograph (pump), DGU-14 degasser, SIL-10AD, CT0-10A, and L-R7A plus chromatopac was used. The filtered black tea syrup extract (10 μ l) was subjected to HPLC analysis using a guard column (4.6 i.d. \times 150 mm; Nomura Chemical, Aichi, Japan) with a guard column (4.0 i.d. \times 100 mm; Develosil ODS-HG-5) and at a flow rate of 0.7 ml/min. The elution was performed using a linear gradient system with two solvents: solvent A, methanol/water/acetic acid (10/88/2, v/v); and solvent B: methanol/water/acetic acid (60/38/2, v/v). The gradient was achieved within 30 min. Absorbance at 280 nm was monitored. Catechin content was determined from the peak area of the samples with reference to calibration of authentic samples.

RESULTS and DISCUSSION

Temperature is one of the important parameters in determination of catechins stability (Chen *et al*, 2001). EGCG was decreased at 90°C (Labbe *et al*, 2008). From catechin and its seven catechins derivatives (EC, EGC, EGCG, GC, GCG, ECG, and CG), five catechin derivatives were detected in black tea syrup: EGC, C, EGCG, EC, and ECG as shown in Table 1.

Table 1. Catechin contents of black tea syrup extract

T(°C)	EGC (μ g/ml)	C (μ g/ml)	EGCG (μ g/ml)	EC (μ g/ml)	ECG (μ g/ml)
30	22.81 \pm 0.3	2.79 \pm 0.02	11.17 \pm 0.15	6.92 \pm 0.66	9.58 \pm 0.16
60	20.35 \pm 3.79	2.49 \pm 0.43	9.93 \pm 1.78	6.96 \pm 1.76	8.58 \pm 1.5
90	21.23 \pm 1.01	2.59 \pm 0.11	10.36 \pm 0.59	6.75 \pm 0.93	8.51 \pm 0.55

Values are mean \pm standard deviation (n = 3)

Some studies have shown that many factors may affect the stability of catechins, including temperature, antioxidant level, and concentrations of other ingredients in tea (Chen *et al.*, 2001; Sang *et al.*, 2006). Higher temperatures for served black tea showed decreased polyphenol content, which decreased the capability of antioxidants against free radicals. Temperature is one of the important parameters in determination of catechins stability (Chen *et al.*, 2001).

Five catechins derivatives were detected in black tea syrup: EGC, C, EGCG, EC, and ECG. Their structures are given in Figure 1. Among the polyphenols, the most abundant compound is EGC. The most important of the catechins are EGC, EGCG, ECG, and EC (Lunter, 1989). As shown, EGC has an important role in this antioxidant activity. Similar results were reported by Muthumani and Senthil (2006), the rate of oxidation follows the order EGC > EGCG > ECG.

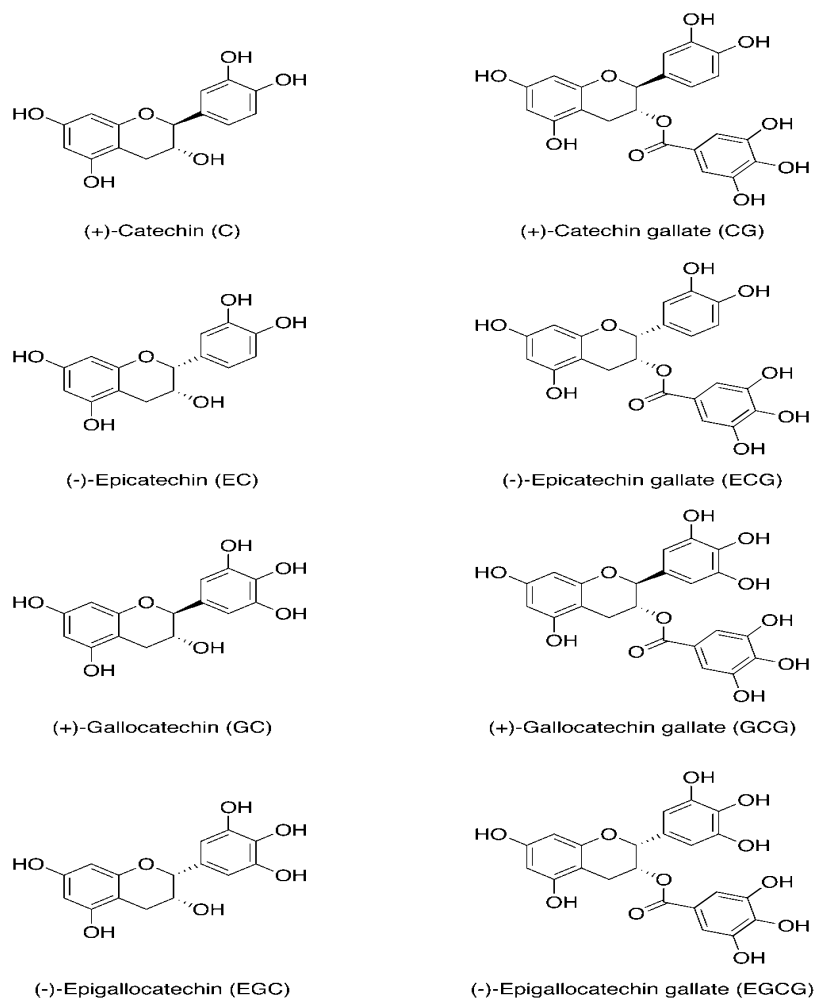


Figure 1. Chemical structures of catechins

Gallocatechin quinones, both in the free and gallated forms, may couple to form a number of bisflavanols that are catechin dimers, without the seven-membered ring that is characteristic of theaflavins. Most of the catechin mass is transformed to the

less structurally defined colored thearubigins in the process of black tea manufacture, with a molecular weight distribution of 1,000-40,000 (Haslam, 2003).

In this study it has been measured the DPPH radical scavenging activity in black

tea syrups prepared at different temperatures using a method widely used to test the ability of compounds to act as a free radical scavenger to evaluate the antioxidative activity. The DPPH radical scavenging activities method gives a strong absorption at 517 nm by visible spectroscopy (purple color) (Molyneux, 2004).

As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, a free radical scavenging antioxidant, the absorption strength is decreased and the resulting decolorization

is stoichiometric with respect to the number of electrons captured (Blois, 1958). Some studies have shown that many factors may affect the stability of catechins, including temperature, antioxidant level, and concentrations of other ingredients in tea (Chen *et al.*, 2001; Sang *et al.*, 2006). The highest DPPH scavenging activities were shown at 30°C. The temperature for brewed black tea syrup is related to the loss of polyphenolic components (Table 1). The highest percentage of DPPH scavenging activity was found at 30°C.

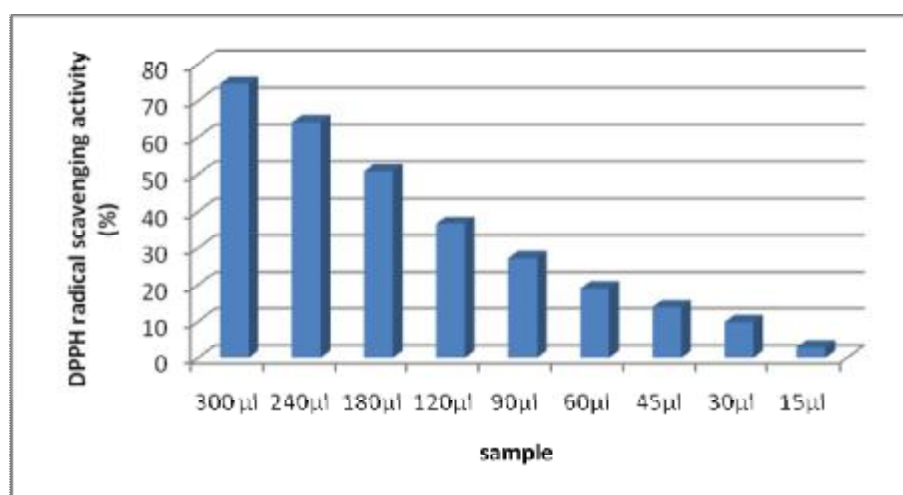


Figure 2. DPPH radical scavenging activity of black tea syrup at 30°C.

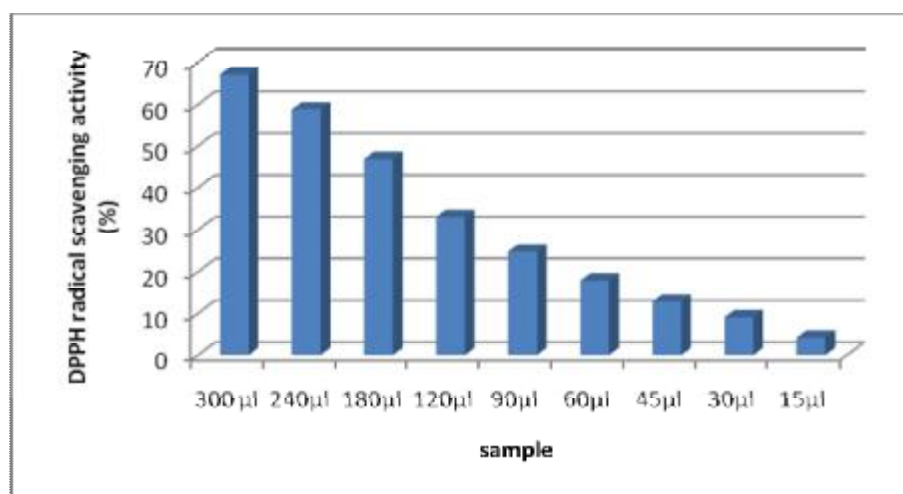


Figure 3. DPPH radical scavenging activity of black tea syrup at 60°C.

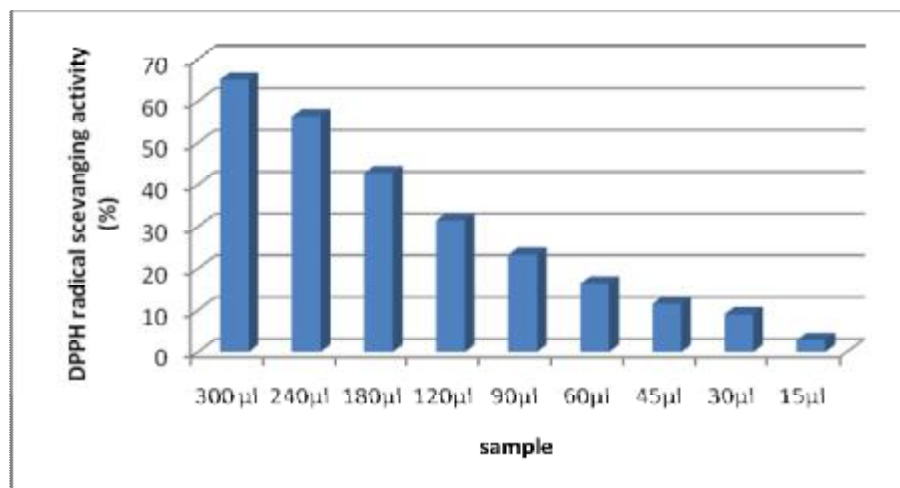


Figure 4. DPPH radical scavenging activity of black tea syrup at 90°C

DPPH radical scavenging activity appeared to depend on the phenolic concentration of the black tea syrup as seen on Figure 2 to 4. The efficiency of some phenol compounds increased the DPPH scavenging activity. Higher temperatures for served black tea showed decreased polyphenol content, which decreased the capability of antioxidants against free radicals. DPPH scavenging activity depends on the phenolic concentration of the fraction. The higher content of total phenolic in black tea syrup might be augmented by the presence of catechins. Catechins, with antioxidative properties, are manifest particularly by their abilities to inhibit and scavenge free radicals.

The molecule of DPPH is characterized as stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical because of its spare electron delocalization over the whole molecule. The delocalization causes a deep violet color with λ_{\max} around 520 nm. When a solution of DPPH is mixed with a substrate acting as hydrogen atom donor, a stable non radical form of DPPH is obtained with simultaneous change of the violet color to pale yellow (Molyneux, 2004).

Antioxidants that are believed to play a very important role in the body's defense system against ROS are of great value in preventing chronic and degenerative diseases. The consumption of plant foods is associated with a reduced risk of chronic diseases, in part because of substances with antioxidant capacity (Day *et al.*, 2004).

CONCLUSION

The results showed that drinking black tea syrup may have health benefits for consumers. Antioxidant activity in the various black tea syrup samples tested (at various temperatures), is correlated with the catechin contents. Consuming black tea syrup at 30°C provided the greatest antioxidant benefit. The antioxidant activity at 30°C was 74,5% for concentration 5 µg/ml (300 µl) and the EGC, C, EGCG, EC and ECG contents were 22,81±0.3, 2.79±0.02, 11,17±0.15, 6.92±0.66 and 9.58±0.16 µg/ml respectively. DPPH radical scavenging activity is considered as a good method which is widely used to assess antioxidant activity within a short time.

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